Serial No. 10/059,521
 Attorney Docket: 6115-007

 Filed: 01/29/2002
 Customer No.: 29,335

 Inventor: Ivan N. Rich
 Confirmation No.: 5794

This Claim Listing replaces all prior versions of claim listings in the application.

Claim Listing:

What is claimed is:

1. (Currently amended) A high-throughput assay method for rapidly determining the proliferative status of a population of primitive hematopoietic cells, the method comprising the steps of:

- (a) incubating a cell population comprising primitive hematopoietic cells in a cell growth medium comprising fetal bovine serum having a concentration of between 0% and about 30%, methyl cellulose having a concentration of between about 0.4% and about 0.7%, transferrin having a concentration of about 0.1 nM and in an atmosphere having between about 3.5% oxygen and about 7.5% oxygen;
- (b) contacting the primitive hematopoietic cell population with a proliferation agent, the proliferation agent selected from the group consisting of a single growth factor, a mix of growth factors, a single cytokine, a mix of cytokines, and combinations thereof;
- (c) (b) contacting the cell population with a reagent capable of generating luminescence in the presence of ATP; and
- (d) (e) detecting luminescence generated by the reagent contacting the cell population, the level of luminescence indicating the amount of ATP in the cell population, wherein the amount of ATP indicates the proliferative status of the primitive hematopoietic cells.
- 2. (Original) The method of Claim 1, wherein the concentration of fetal bovine serum is between about 0% and 10%.

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3. (Original) The method of Claim 1, wherein the concentration of methyl cellulose is about 0.7%.

- 4. (Original) The method of Claim 1, wherein the concentration of oxygen in the atmosphere is about 5%.
- 5. (Canceled).
- 6. (Currently amended) The method of Claim $\underline{1}$ [[5]], further comprising the step of generating a cell population substantially enriched in hematopoietic stem cells.
- 7. (Currently amended) The method of Claim 1 [[5]], further comprising the step of generating a cell population substantially enriched in at least one hematopoietic progenitor cell lineage.
- 8. (Original) The method of Claim 1, wherein the primitive hematopoietic cells are hematopoietic stem cells.
- 9. (Original) The method of Claim 1, wherein the primitive hematopoietic cells are hematopoietic progenitor cells.
- 10. (Original) The method of Claim 1, wherein the population of primitive hematopoietic cells comprises hematopoietic stem cells and hematopoietic progenitor cells.
- 11. (Original) The method of Claim 1, wherein the primitive hematopoietic cells are primary hematopoietic cells.

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12. (Original) The method of Claim 11, wherein the primary hematopoietic cells are isolated from an animal tissue selected from the group consisting of peripheral blood, bone marrow, umbilical cord blood, yolk sac, fetal liver and spleen.

- 13. (Original) The method of Claim 12, wherein the animal tissue is obtained from a human.
- 14. (Original) The method of Claim 12, wherein the animal tissue is obtained from a mammal.
- 15. (Original) The method of Claim 14, wherein the mammal is selected from the group consisting of cow, sheep, pig, horse, goat, dog, cat, non-human primates, rodents, rabbit and hare.
- 16.-17. (Canceled).
- 18. (Original) The method of Claim 11, wherein the primary hematopoietic stem cells are isolated from peripheral blood.
- 19. (Previously presented) The method of Claim 1, further comprising the step of selecting a differentially distinguishable subpopulation of primitive hematopoietic cells from the population of primitive hematopoietic cells, wherein the differentially distinguishable subpopulation of primitive hematopoietic cells is defined by cell surface markers thereon.

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20. (Previously presented) The method of Claim 19, wherein the step of selecting a differentially distinguishable subpopulation of primitive hematopoietic cells comprises the steps of:

- (a) contacting the population of primitive hematopoietic cells with at least one cell surface marker indicator, wherein the at least one cell surface marker indicator is a specific ligand for a cell surface marker that differentially distinguishes a subpopulation of primitive hematopoietic cells, and whereby the at least one cell surface marker indicator binds to the cell surface marker; and
- (b) selectively isolating the subpopulation of primitive hematopoietic cells binding the at least one indicator.
- 21. (Original) The method of Claim 19, wherein the cell surface marker is selected from the group consisting of CD3, CD4, CD8, CD34, CD90 (Thy-1) antigen, CD117, CD38, CD56, CD61, CD41, glycophorin A and HLA-DR, AC133 defining a subset of CD34⁺ cells, CD 19, and HLA-DR.
- 22. (Original) The method of Claim 19, wherein the cell surface marker is CD34⁺.
- 23. (Previously presented) The method of Claim 20, wherein the differentially distinguishable subpopulation of primitive hematopoietic cells is selectively isolated by magnetic bead separation.
- 24. (Previously presented) The method of Claim 20, wherein the differentially distinguishable subpopulation of primitive hematopoietic cells is selectively isolated by flow cytometry and cell sorting.

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25. (Previously presented) The method of Claim 1, wherein the population of primitive hematopoietic cells comprises at least one stem cell lineage selected from the group consisting of colony-forming cell-blast (CFC-blast), high proliferative potential colony forming cell (HPP-CFC), and colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM).

- 26. (Previously presented) The method of Claim 1, wherein the population of primitive hematopoietic cells comprises at least one hematopoietic progenitor cell lineage selected from the group consisting of granulocytemacrophage colony-forming cell (GM-CFC), megakaryocyte colony-forming cell (CFC-mega), macrophage colony-forming cell (M-CFC), granulocyte colony forming cell (G-CFC), burst-forming unit erythroid (BFU-E), colony-forming unit-erythroid (CFU-E), colony-forming cell-basophil (CFC-Bas), colony-forming cell-eosinophil (CFC-Eo), colony-forming cell-megakaryocyte (CFC-Mega), B cell colony-forming cell (B-CFC), and T cell colony-forming cell (T-CFC).
- 27. (Original) The method of Claim 1, wherein the reagent capable of generating luminescence in the presence of ATP comprises luciferin and luciferase.
- 28. (Currently amended) The method of Claim 1 [[5]], wherein the proliferation agent is selected from the group consisting of erythropoietin, granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor, macrophage colony stimulating factor, thrombopoietin, stem cell factor, interleukin-1, interleukin-2, interleukin-3, interleukin-6, interleukin-7, interleukin-15, Flt3L, leukemia inhibitory factor, insulin-like growth factor, insulin, and combinations thereof.

29-30. (Canceled).

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31. (Previously presented) The method of Claim 5, wherein the proliferation agent is erythropoietin, granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor, stem cell factor, interleukin-3, interleukin-6, and optionally Flt3L, and wherein the proliferation agent generates a cell population substantially enriched in hematopoietic colony-forming cell-granulocyte, erythroid, macrophage, megakaryocyte (CFC-GEMM) stem cells.

32.-41. (Canceled).

- 42. (Original) The method of Claim 1, further comprising the step of identifying a population of primitive hematopoietic cells having a proliferative status suitable for transplantation into a recipient patient.
- 43. (Previously presented) The method of Claim 1, wherein the population of primitive hematopoietic cells comprises a target cell population, and further comprising the steps of:
 - (i) contacting the target cell population with a test compound; and
 - (ii) determining the ability of the test compound to modulate the proliferation, and optionally differentiation, of the target cell population.

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44. (Previously presented) The method of Claim 1, wherein the population of primitive hematopoietic cells comprises a plurality of target cell populations, and further comprising the steps of:

- contacting the plurality of target cell populations with at least one (i) test compound;
- (ii) determining the ability of the at least one test compound to alter the proliferation of the target cell population by comparing the proliferative status of the plurality of target cell populations with the proliferative status of a target population of primitive hematopoietic cells not in contact with the test compound; and
- identifying the at least one test compound modulating the (iii) proliferative status of a target cell population.

45.-56. (Canceled).

- 57. (Currently amended) The method of Claim 59 [[1]], wherein the transferrin is an iron-saturated transferrin.
- 58. (Previously presented) The method of Claim 57, wherein the ironsaturated transferrin is a human or bovine iron-saturated transferrin.
- (New) The method of Claim 1, wherein the cell growth medium further 59. comprises transferrin.
- 60. (New) The method of Claim 59, wherein the transferrin has a concentration of about 0.1 nM.

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